A New Method for Lignin Characterization (Preliminary Report)

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Introduction

Unlike other natural polymers, such as proteins, polysaccharides and nucleic acid which have interunit linkages susceptible to enzymic and chemical hydrolyses, lignin contains non-cleavable carbon-carbon and diphenyl ether bonds between its building units. So only a part of lignin, where units are linked by β-O-4 or α-O-4 ethers, can be degraded and characterized. If uncondensed linkages are cleaved selectively and completely, the characterization of the degradation products such as monomers, dimers and trimers will provide valuable structural information about the initial lignin. At present, thioacidolysis is the preeminent method for quite selectively cleaving ethers to produce analyzable monomers and dimers. That procedure, while valuable, is not a trivial technique to perform and has significant drawbacks, not the least of which is the foul stench of the required reagents.

Here we report on a completely new approach to complete ether cleavage in lignin to provide a relatively simple, efficient method for lignin analysis. Our results with some representative lignin models, isolated lignin samples, and plant materials demonstrate the high selectivity, high yield of the degradation products and simplicity in operation as well as the usefulness for lignin structure characterization. Regrettably, the procedure is not completely developed at this point but, as it is intended for release at the March National American Chemical Society Meeting in New Orleans, belongs here this year.

Experimental

[Not the final procedure!] To 10 mg of lignin model or lignin sample in 10 ml flask is added 2 ml of stock reagent (AcBr in acetic acid). The mixture is kept at room temperature with gentle stirring overnight. After removal of solvent by rotary evaporator(< 45 °C), the residue is dissolved in 2 ml of dioxane to which is added 50 mg of Zn dust while it is well stirred, followed by addition of 2 ml of 0.8 N HCl 4:1 dioxane water solution. This mixture is stirred for 15 min. After addition of internal standard (methyl 3,5-dimethoxybenzonate) the mixture is poured into ethyl acetate (20 ml) and washed with 3% of HCl twice, sat. NH_4Cl once and dried over $MgSO_4$. After evaporation the residue is dissolved in 3.0 ml of acetic acid to which 10 mg of $Pd/BaSO_4$ is added. This mixture is stirred for 2 h under hydrogen (balloon), then acetic acid is removed by evaporation and 1 ml of pyridine/Ac_2O (1:1) is added. After standing for 45 min. and normal work-up, the residue is dissolved in 200 µl of methylene chloride and 1 µl of this solution is injected into GC for analysis.

Table 1. Yields of the main monomers by the method from lignin models and MWLs.

Model or Lignin Sample	Yield (% W)[a]	Molar Yield[a] (mmole/g)	Relative ratio H : G : S
Guaiacyl β-O-4	95[b]	——	——
Syringyl β-O-4	91[b]	——	——
Pine	19.14	722	1 : 13.5 : 0
Willow	21.84	904	1 : 11.7 : 12.6
Kenaf	30.50	1050[c]	trace : 1 : 5
Bromegrass	20.35	781	1 : 19.6 : 15.6
Alfalfa	13.62	570	1 : 10.4 : 4.1
Corn	12.96	351	1 : 6 : 5.7

[a]The yield is based on whole MWLs.
[b]Theoretical yield.
[c]The molar yield from kenaf lignin by thioacidolysis is 967mmole/g.

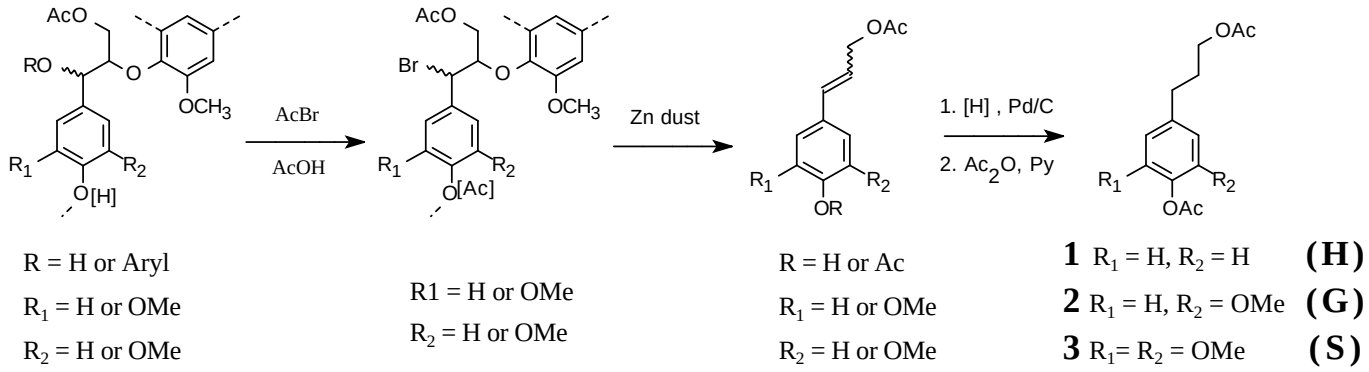

*Figure 1. AcBr/Zn-based method for cleavage of ether linkages in lignins to produce monomers **1**-**3**.*

Results and Discussion

As shown in Figure 1, AcBr/Zn degradation method includes three key steps: 1. bromination and acetylation with AcBr, 2. reductive elimination with Zn dust, and 3. hydrogenation and acetylation. The reaction of lignin with AcBr in acetic acid results in complete dissolution of lignin (and other wall components) and formation of bromo-acetylated lignin derivative which has β-bromoaryl ether skeleton. In the next step, the β-bromo ethers are cleaved by reductive elimination with Zn forming pairs of cinnamyl acetate isomers. It is necessary to have the last step to reduce the numbers of degradation monomers and make GC quantitation simpler. Thus the monomers derived from this method are essentially **1-3** (see Figure 2). From the table below we can see that β-ether linkage in model compounds, representatives of lignin major substructures, is efficiently cleaved by this method and monomers are recovered in high yield (95% and 91% of theoretical). When isolate lignins are used in this method, substantial amounts of degraded monomers are obtained; the yield of the monomers from kenaf lignin is higher than from thioacidolysis.

In summary, we have developed a new method which selectively cleaves ether linkages in lignin, a useful lignin characterization method. Its relative simplicity, selectivity (cleanliness) and the use of relatively innocuous reagents, may well challenge the current thioacidolysis procedure.

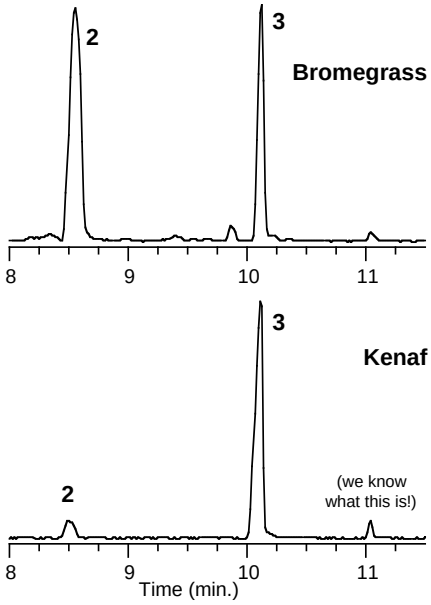

Figure 2. GC-MS total ion chromatograms of the products from two isolated lignins.